Origin and Early Evolution of Angiosperms

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Contributions from paleobotany, phylogenetics, genomics, developmental biology, and developmental genetics have yielded tremendous insight into Darwin's "abominable mystery"—the origin and rapid diversification of the angiosperms. Analyses of morphological and molecular data reveal a revised "anthophyte clade" consisting of the fossils glossopterids, *Pentoxylon,* Bennettitales, and *Caytonia* as sister to angiosperms. Molecular estimates of the age of crown group angiosperms have converged on 140–180 million years ago (Ma), older than the oldest fossils (132 Ma), suggesting that older fossils remain to be discovered. Whether the first angiosperms were forest shrubs (dark-and-disturbed hypothesis) or aquatic herbs (wet-and-wild hypothesis) remains unclear. The near-basal phylogenetic position of Nymphaeales (water lilies), which may include the well-known fossil *Archaefructus*, certainly indicates that the aquatic habit arose early. After initial, early "experiments," angiosperms radiated rapidly (≤5 million years [Myr]), yielding the five lineages of Mesangiospermae (magnoliids and Chloranthaceae as sisters to a clade of monocots and eudicots + Ceratophyllaceae). This radiation ultimately produced approximately 97% of all angiosperm species. Updated estimates of divergence times across the angiosperms conducted using nonparametric rate smoothing, with one or multiple fossils, were older than previous reports, whereas estimates using PATHd8 were typically younger. Virtually all angiosperm genomes show evidence of whole-genome duplication, indicating that polyploidy may have been an important catalyst in angiosperm evolution. Although the flower is the central feature of the angiosperms, its origin and subsequent diversification remain major questions. Variation in spatial expression of floral regulators may control major differences in floral morphology between basal angiosperms and eudicot models.

Key words: **phylogeny; divergence; floral evolution; genome evolution**

Introduction

Angiosperms represent one of the greatest terrestrial radiations. The oldest fossils date from the early Cretaceous (Friis *et al.* 2006), 130–136 million years ago (Ma), followed by a rise to ecological dominance in many habitats before the end of the Cretaceous. Darwin referred to the origin of the angiosperms as "an abominable mystery" in a well-known quotation taken from a letter to J.D. Hooker. Through contributions from paleobotany, phylogenetics, classical developmental biology, and modern developmental genetics (evo–devo), tremendous progress has recently been made in elucidating the origin and diversification of the angiosperms. We attempt to synthesize and summarize many of these recent developments, noting their importance to the rapidly changing angiosperm paradigm. We also provide a possible road map for future research.

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Fossils

Paleobotany has at least three crucial roles to play in resolving the origin and early diversification of the flowering plants and addressing the following questions: (1) What lineage is ancestral to the angiosperms? (2) What were the first angiosperms, and how have characters evolved? Fossil discoveries and the analysis of existing data have made significant contributions to our understanding of the possible ancestors of flowering plants (see most recently Friis *et al.* 2007), as well as to the early diversification of angiosperms. (3) When did the major clades of angiosperms diversify, on the basis of fossil calibrations and molecular-based methods?

Ancestors of Flowering Plants

The closest relatives of angiosperms remain a mystery. Phylogenetic analysis of morphological features conducted in the 1980s suggested that Gnetales were the closest living relatives of angiosperms (e.g., Crane 1985; Doyle & Donoghue 1986). Cladistic analyses of extant and fossil taxa recovered a clade of Bennettitales, *Pentoxylon*, Gnetales, and angiosperms (Crane 1985; Doyle & Donoghue 1986). Doyle and Donoghue (1986) named this clade the "anthophytes" in reference to the flowerlike reproductive structures in all members. The anthophyte hypothesis, that angiosperms belong to this clade and are possibly sister to the gnetophytes, concomitantly had a profound effect on views of the evolution of the angiosperms. For example, acceptance of the anthophyte hypothesis stimulated the reinterpretation of character evolution (Frohlich 1999; Donoghue & Doyle 2000), including the origin of the carpel and double fertilization (Friedman 1994; Doyle 1998).

Molecular phylogenetic studies, however, later revealed that Gnetales are not the sister group to the angiosperms but that extant gymnosperms form a clade, with Gnetales most likely associated with conifers (e.g., Qiu *et al.* 1999; Soltis *et al.* 1999a; Chaw *et al.* 2000; Magallón & Sanderson 2002; Burleigh & Mathews 2004). Furthermore, there are no known fossils representing unequivocal stem group angiosperms (i.e., angiosperms that clearly attach below the basal node leading to *Amborella*, Nymphaeales, and all other living angiosperms). Hence, determining the closest relatives of angiosperms is a major challenge.

The demise of the anthophyte hypothesis profoundly altered views of angiosperm origins; accompanying this demise is a need for alternatives. If no extant gymnosperm is sister to angiosperms, what fossil lineages, if any, are closely related to flowering plants? Doyle (2001) and Soltis *et al.* (2005) conducted a series of analyses of morphological and molecular data sets involving extant taxa, as well as fossils, to reassess the closest relatives of the angiosperms. These analyses yielded similar results and revealed a revised "anthophyte clade." In this clade *Caytonia* is sister to angiosperms, followed by Bennettitales, *Pentoxylon,* and glossopterids (Fig. 1). Hence, fossils such as *Caytonia* and Bennettitales may be the closest relatives of angiosperms (Fig. 1). However, the recent discovery that gnetophytes and Bennettitales share features (Friis *et al.* 2007) suggests that further attention be given to circumscribing and evaluating a revised anthophyte clade. Although these analyses are useful, one of the biggest remaining challenges facing evolutionary biologists is ascertaining the fossil lineages that represent the closest relatives of angiosperms. Do Caytoniales and Bennettitales actually represent these lineages? Are there key fossil links that remain to be discovered?

The Earliest Angiosperms: Fossils

There are two highly divergent views on the general habit of the earliest angiosperms: woody and terrestrial or herbaceous and aquatic. This has actually been a longstanding debate (reviewed in Soltis *et al.* 2005). The hypothesis that the earliest angiosperms were woody is supported by the fact that most basal

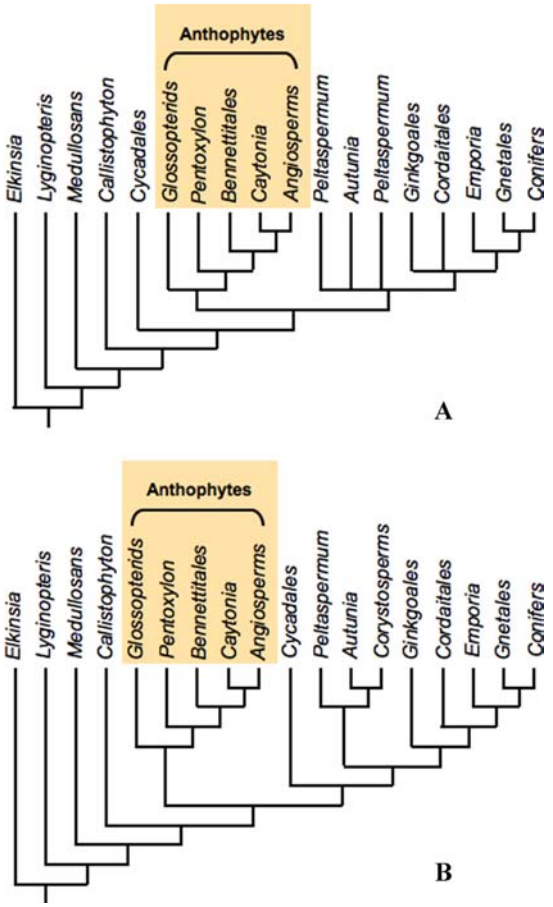

Figure 1. Revised views of the phylogeny of seed plants showing putative closest relatives of angiosperms obtained by using both the morphological matrix of Doyle (1996) and molecular data (reviewed in Soltis *et al.* 2005). A revised "anthophyte" clade is depicted. (**A**) Tree modified from Soltis *et al.* (2005), in which molecular data for seed plants are combined with the morphological matrix of Doyle (1996). (**B**) Tree modified from Doyle (2001), in which a molecular constraint is used, placing Gnetales with other extant gymnosperms.

angiosperms are woody, and all gymnosperms are woody, as are the fossil lineages that are considered most closely related to angiosperms (e.g., Caytoniales, Bennettitales; see earlier discussion). *Amborella*, the sister to all other living angiosperms, is woody, as are members of Austrobaileyales, another early-branching lineage of living angiosperms.

An aquatic origin of angiosperms is supported by the fact that several of the earliest known fossil angiosperms were aquatic. *Archaefructus* represents perhaps the oldest, most complete angiosperm fossil (Sun *et al.* 2002); it is estimated to be approximately 115–125 million years (Myr) old. On the basis of morphology, it clearly was aquatic. The phylogenetic placement of the fossil *Archaefructus* as sister to all extant angiosperms (Sun *et al.* 2002), plus the near-basal phylogenetic position of extant Nymphaeales (water lilies, below), lends support to the view that the aquatic habit arose early in angiosperms and that perhaps the earliest angiosperms were aquatic. However, later analyses (e.g., Friis *et al.* 2003) questioned the placement of *Archaefructus* as sister to all extant angiosperms; some analyses of Friis *et al.* placed *Archaefructus* with water lilies. The recent discovery that Hydatellaceae are part of the water lily clade (Saarela *et al.* 2007), and hence a new branch near the base of the angiosperms, greatly increases the morphological diversity encompassed by the Nymphaeales clade. This finding raised the possibility that Nymphaeales were once much more diverse, and could have encompassed *Archaefructus*, as well as other now-extinct lineages (Doyle, submitted). Hence, it now seems prudent to keep an open mind regarding the placement of *Archaefructus*—it represents well the difficulty in placing fossil lineages that are morphologically distinct, with no clear synapomorphies with extant taxa.

Another early angiosperm fossil (unnamed) was considered a possible water lily relative by Friis *et al.* (2001). This fossil is dated at approximately 125–115 Myr old and was used as evidence to support the antiquity of the Nymphaeales lineage. This putative ancient water lily fossil is therefore extremely important in discussions of the diversification of Nymphaeales, as well as the angiosperms. A phylogenetic analysis, using the morphological data set of Les *et al.* (1999) for extant Nymphaeales, placed the fossil as sister to Nymphaeales; synapomorphies with extant Nymphaeales included a syncarpous gynoecium, a perigynous or epigynous perianth, and

a central protrusion of the floral apex between the carpels. However, neither of the last two characters is found consistently throughout Nymphaeales (Friis *et al.* 2001). The unnamed fossil exhibits features shared by both Nymphaeales and *Illicium* (Austrobaileyales) (Friis *et al.* 2001; Gandolfo *et al.* 2004; Yoo *et al.* 2005) and occurs in a site with abundant fossil seeds attributed to Illiciales (Friis *et al.* 2001). The recent molecular analyses and dating experiments of Yoo *et al.* (2005) raise the possibility that the unnamed fossil of Friis *et al.* may have actually been part of an ancient assemblage that included Nymphaeales and Austrobaileyales. That is, the unnamed fossil probably occupies a deeper place on the angiosperm tree than the branch leading to water lilies. Endress (2008) recently reached the same conclusion on the basis of a reexamination of the morphology of *Microvictoria* (Gandolfo *et al.* 2004)—the fossil differs in key features from extant genera of Nymphaeaceae.

This unnamed fossil of Friis *et al.* (2001) and *Archaefructus* certainly indicate that the aquatic habit arose early in angiosperm evolution. This view is further supported by the near-basal placement of Nymphaeales (water lilies) in phylogenies of extant taxa. However, there are undoubtedly earlier, as yet undiscovered, angiosperm fossils. In this regard, molecular estimates for the age of the angiosperms are converging; most recent estimates are in the range of 140–180 Ma (Bell *et al.* 2005), suggesting an age for flowering plants that is substantially older than the date of 132 Ma on the basis of the known fossil record. These molecular estimates suggest, in fact, that the earliest angiosperms may have arisen in the late Jurassic, rather than the early Cretaceous, and that the oldest angiosperm fossils are still undiscovered.

Phylogenetics

Resolving the Big Picture

One of most exciting recent developments in the study of angiosperm evolution has been the enormous progress made in elucidating angiosperm evolutionary history (reviewed in Judd & Olmstead 2004; Soltis & Soltis 2004; Soltis *et al.* 2005). We provide a summary tree of our current understanding of angiosperm relationships (Fig. 2). Phylogenetic analyses based on an ever-increasing number of gene sequences have clearly and unequivocally established the first branches of extant angiosperm diversity. Many studies have revealed strong support for the sister relationship of Amborellaceae, followed by Nymphaeales, and then Austrobaileyales to all other extant angiosperms (Mathews & Donoghue 1999, 2000; Parkinson *et al.* 1999; Qiu *et al.* 1999, 2000, 2005; Soltis *et al.* 1999b, 2000, 2004; Barkman *et al.* 2000; Graham & Olmstead 2000; Graham *et al.* 2000; Soltis *et al.* 2000; Zanis *et al.* 2002,2003; Borsch *et al.* 2003; Hilu *et al.* 2003; Kim *et al.* 2004; Stefanović *et al.* 2004; Leebens-Mack *et al.* 2005; Stefanović *et al.* 2005; Jansen *et al.* 2007; Moore *et al.* 2007). In most analyses, the monotypic *Amborella* is followed by the water lilies or Nymphaeales (which comprise Nymphaeaceae) and Cabombaceae (APG II 2003) and Hydatellaceae (Saarela *et al.* 2007); although a few investigations placed Amborellaceae plus Nymphaeales as sister to all other angiosperms (reviewed in Soltis *et al.* 2005), recent analyses involving complete or nearly complete plastid genome sequences have placed *Amborella* alone as sister to all other extant angiosperms (e.g., Jansen *et al.* 2007; Moore *et al.* 2007). Although this now appears to be the favored topology, it should be confirmed using nuclear sequence data.

An exciting recent molecular finding is the placement of *Hydatella* (Hydatellaceae) in the Nymphaeales clade (Saarela *et al.* 2007). Hydatellaceae had been placed in the monocots (Poales), but molecular data revealed instead that the family is part of the early-diverging angiosperm clade Nymphaeales with high support values. Hence, this is a dramatic phylogenetic shift. This result is important in that it greatly expands the morphological diversity encompassed by the Nymphaeales. For example,

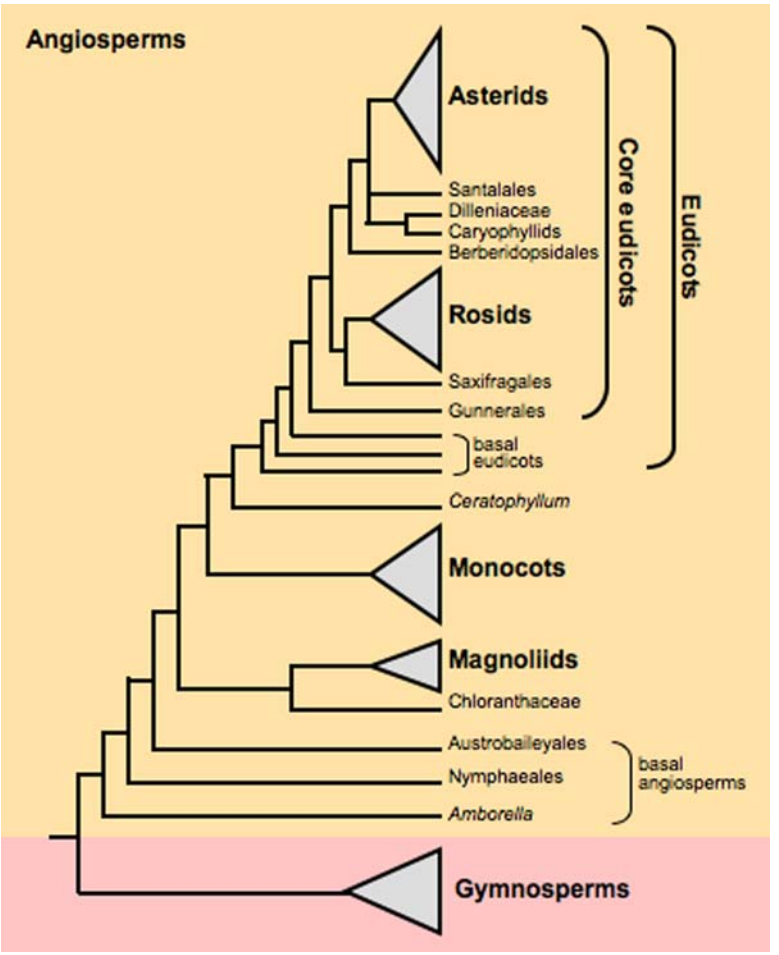

Figure 2. Summary topology of current view of deep-level angiosperm relationships. This topology is based on the analyses of the three-gene, 567-taxon data set (Soltis *et al.* 2000, 2007a) with modifications based on the recent analyses of nearly complete plastid genome data sets (Jansen *et al.* 2007; Moore *et al.* 2007).

Rudall *et al.* (2007) proposed that the most plausible interpretation of the reproductive units is that each such unit represents an aggregation of reduced, unisexual, apetalous flowers; this type is different from flowers of Nymphaeales (Rudall *et al.* 2007). However, *Hydatella* has ascidiate carpel development, consistent with placement in Nymphaeales. The placement of *Hydatella* within Nymphaeales raises the possibility that this clade was once far more diverse morphologically and increases the plausibility that the morphology of *Archaefructus* could be encompassed by this clade, as originally proposed by Friis *et al.* (2003; see also Doyle, submitted).

After *Amborella* and Nymphaeales, an Austrobaileyales clade of Illiciaceae, Austrobaileyaceae, and Trimeniaceae is the subsequent sister group to all remaining angiosperms. Whereas these basalmost branches are well supported and resolved, relationships among the remaining major lineages of angiosperms have been unclear and difficult to resolve, with three-, five-, nine-, and even 11-gene data sets (see references above). These remaining angiosperms, or Mesangiospermae (Cantino *et al.* 2007), are magnoliids, Chloranthaceae, monocots, Ceratophyllaceae, and eudicots. Analyses of multigene data sets with adequate taxon sampling have provided strong support for the

monophyly of each of these clades of angiosperms. However, different analyses have depicted various relationships among these five clades, with only weak support.

In contrast to earlier studies, recent analyses based on complete or nearly complete plastid genome sequences appear to resolve relationships among these five lineages of Mesangiospermae. After the grade of *Amborella*, Nymphaeales, and Austrobaileyales, Chloranthaceae and magnoliids may form a clade that is sister to a large clade of monocots and Ceratophyllaceae plus eudicots (Moore *et al.* 2007) (Fig. 2). The clade of monocots as sister to the eudicot+*Ceratophyllum* clade is strongly supported (Moore *et al.* 2007). Jansen *et al.* (2007) also found these relationships, except that their initial analysis did not include *Ceratophyllum*. The first three living branches were relatively easy to recover in phylogenetic analyses, a result reported in some of the initial multigene analyses of angiosperms (e.g., Soltis *et al.* 1999, 2000; Qiu *et al.* 1999; Parkinson *et al.* 1999; Mathews & Donoghue 1999, 2000). These results suggested a more gradual diversification process (or greater extinction) at the base of the angiosperm tree. However, after some initial early "experiments" in angiospermy, the angiosperms rapidly radiated, yielding the five lineages of Mesangiospermae. This is an exciting example of an important molecular insight into Darwin's "abominable mystery." The fossil record certainly supports the presence of many diverse lineages early in angiosperm evolution (e.g., Friis *et al.* 1994, 1999, 2000, 2001), but phylogenetic analyses of extant angiosperms indicate that the radiation responsible for nearly all extant angiosperm diversity was not associated with the origin of the angiosperms but occurred after the diversification of *Amborella*, Nymphaeales, and Austrobaileyales (Mathews & Donoghue 1999; Soltis *et al.* 1999b). The clades composing the Mesangiospermae (monocots, Ceratophyllaceae, eudicots, Chloranthaceae, and magnoliids) represent the "big bang" of angiosperm evolution—they diversified rapidly (within only a few million years); therefore, resolving relationships among these clades has been difficult.

Recent analyses (e.g., Moore *et al.* 2007; Jansen *et al.* 2007; Jian *et al.*, 2008) demonstrate that with enough sequence data (20,000 or more base pairs), the remaining, problematic, deep-level problems in the flowering plants can also be resolved. Especially promising are analyses involving complete sequencing of the plastid genome (e.g., Jansen *et al.* 2007; Moore *et al.* 2007), particularly given the current ease of complete plastid genome sequencing with new sequencing technologies (e.g., Moore *et al.* 2006). An alternative method that is promising, and much less costly, is the complete sequencing of the slowly evolving inverted repeat of the plastid genome (Jian *et al.*, 2008), which is readily accomplished in most angiosperm groups by using the polymerase chain reaction–based ASAP (amplification, sequencing and annotation of plastomes; Dhingra & Folta 2005) method.

Implications of Phylogeny for the Earliest Angiosperms

A well-resolved phylogeny for extant angiosperms has been used as a framework in an effort to reconstruct the habit, as well as other morphological features, of the earliest flowering plants (APG II 2003; Soltis *et al.* 2005). As noted, *Amborella*, which is sister to all other extant angiosperms, is woody, as are Austrobaileyales. Anatomical and physiological features of *Amborella* (which lacks vessel elements) and Austrobaileyales (which have "primitive" vessel elements considered intermediate between tracheids and vessel elements) (Carlquist & Schneider 2001, 2002) prompted the hypothesis that the earliest angiosperms were understory shrubs, perhaps without vessel elements, and with lower transpiration and stem water movement than is typical of most extant angiosperms (Feild *et al.* 2000, 2003, 2004). Such plants occupied habitats referred to as "dark and disturbed" and prompted the hypothesis that the first angiosperms were

understory shrubs living in partially shaded habitats that depended on canopy disturbance to open up new sites for colonization. Outgroup comparisons favor a woody habit for the first angiosperms. All other "anthophytes" are woody; this includes extant gymnosperms, as well as those fossils typically considered close relatives of angiosperms (e.g., Caytoniales, Bennettitales).

However, the strictly aquatic water lily lineage (Nymphaeales) follows *Amborella* as the subsequent sister to all other flowering plants. As a result of the near-basal placement of Nymphaeaceae, the habit of the first flowering plants is reconstructed as equivocal on the basis of analyses of extant taxa (Soltis *et al.* 2005). Nonetheless, the early appearance of the water lily lineage, together with the discovery that some of the oldest angiosperm fossils are aquatic (e.g., *Archaefructus*), reinforced the hypothesis that the earliest angiosperms may have been aquatic—that is, "wet and wild" (D. Dilcher, pers. comm.; see Coiffard *et al.* 2007).

Molecular Data and Divergence Times

Dating Origin of the Angiosperms

In an attempt to estimate the timing of angiosperm origins, various authors have used a variety of different molecular data sets and estimation procedures. Although the oldest angiosperm fossils date to 132 Ma, molecular estimates have been somewhat older. Molecular estimates have dated angiosperm origins to the Lower Jurassic (175–200 Ma; Sanderson 1997; Sanderson & Doyle 2001; Wikström *et al.* 2001; Bell *et al.* 2005; Magallón & Sanderson 2005), to the Triassic (200–250 Ma; Sanderson & Doyle 2001; Magallón & Sanderson 2005), or even the Paleozoic (300 or 350 Ma; e.g., Ramshaw *et al.* 1972; Martin *et al.* 1989). However, in recent years, studies estimating the age of crown group angiosperms appear to be converging on estimates of between 140 and 180 Ma (Sanderson *et al.* 2004; Bell *et al.* 2005; Moore *et al.* 2007). This apparent convergence in age estimation is probably due to the increased number of characters used, as well as the use of new "relaxed clock" methods that allow rates to vary across the tree (e.g., penalized likelihood, PATHd8, and Bayesian methods). Also, recent studies have used multiple fossils as either calibrations or maximum and minimum age constraints.

To date, the most comprehensive divergence time analysis for the angiosperms, for taxon sampling, is that of Wikström *et al.* (2001). The results from Wikström *et al.* (2001) have subsequently been used as a temporal framework for many ecological studies (e.g., Slingsby & Verboom 2006; Vamosi *et al.* 2006; Edwards *et al.* 2007; Webb *et al.* 2007), as well as "external" calibration points for subsequent divergence time analyses of groups that may lack reliable fossils (e.g., Crayn *et al.* 2006; Park *et al.* 2006). Although a landmark study, the analysis of Wikström *et al.* suffered in several methodological ways that were unavoidable at the time because the necessary analytical tools were lacking, namely, reliance on one calibration point and the use of nonparametric rate smoothing (NPRS; a method that has been shown to be a biased estimator of clade ages).

Here we have reanalyzed the data set used by Wikström *et al.* by using multiple calibration points and age constraints (21 total; Table 1) and a tree topology (Fig. 3) inferred from a recent Bayesian analysis (Soltis *et al.* 2007). To make our results as comparable as possible to those of Wikström *et al.* (2001), we used their fossil calibration point as a fixed age in all analyses and inferred ages by using NPRS. We also used PATHd8 (Britton *et al.* 2007), a recently proposed relaxed-clock method to estimate divergence times across angiosperms. Like Wikström *et al.* (2001), we calculate a range of ages on the basis of branch length estimates from parsimony and maximum likelihood. Under the maximum likelihood criterion, a general-time-reversible model of sequence

TABLE 1. Multiple Calibration Points and Age Constraints Used in Divergence Time Estimations

Organism group	Age (Myr)	MRCA[a] of:
Altingia (Altingiaceae)	88.5–90.4	*Altingia* and *Paeonia*
Hamamelidaceae	84–86	*Daphniphyllum* and *Itea*
Cercidiphyllaceae	65–71	*Cercidiphyllum* and *Crassula*
Divisestylus (Iteaceae)	89.5–93.5	*Ribes* and *Itea*
Ailanthus (Simaroubaceae/Rutaceae/Meliaceae)	50	*Ailanthus* and *Swietenia*
Burseraceae/Anacardiaceae	50	*Bursera* and *Schinus*
Parbombacaceoxylon (Malvales s.l.)	65.5–70.6	*Thymea* and *Bombax*
Bedelia (Fagales)	84	*Cucumis* and *Carya*
Paleoclusia (*Clusia*/*Hypericum*)	89–90	*Dicella* and *Mesua*
Illiciospermum (Illiciales)	89–99	*Illicium* and *Schisandra*
Diplodipelta	36	*Valeriana* and *Dipsacus*
Angiosperm Crown Group	131.8 (min)	*Amborella* and *Valeriana*
Eudicots (crown group)	125	*Ranunculus* and *Valeriana*
Virginianthus (Calycanthaceae)	98–113	*Calycanthus* and *Idiospermum*
Unnamed (Chloranthaceae)	98–113	*Hedyosmum* and *Chloranthus*
Perisyncolporites (Malpighiales)	49	*Dicella* and *Malpighia*
Pseudosalix (Malpighiales)	48	*Idesia* and *Populus*
Cornales	86	*Cornus* and *Nyssa*
Platanaceae	98–113	*Platanus* and *Placospermum*
Buxaceae	98–113	*Didymeles* and *Buxus*
Bignoniaceae	49.4	*Catalp*a and *Verbena*
Bignoniaceae	35	*Catalpa* and *Campsis*

[a]MRCA, most recent common ancestor.

evolution was used. To accommodate among-site rate variation, we used a discrete gamma (Γ) distribution with four rate categories and accounted for the proportion of invariable sites (I) in the substitution model (Fig. 3).

The results of these analyses for some of the major early angiosperm groups are summarized in Table 2. In almost all cases, our NPRS dates, with either one or multiple fossils, were older than those of Wikström *et al.* (2001), potentially because we are using a slightly different tree topology (Soltis *et al.* 2007a), Likewise, across the nodes examined here, PATHd8 provided younger age estimates under each fossil treatment.

The mean absolute difference across all nodes using these methods was approximately 5 Myr, with nearly all differences being older in the multiple-calibration analysis than the single-calibration analysis. This finding is potentially significant given that the average bootstrap estimate of standard errors was less than these values. However, the difference at any specific node was greater than 10 Myr for only three of the nodes. One explanation for the differences is that many of the minimum age constraints used in our study (seven of 20) were older than the ages inferred by Wikström *et al.* (2001), pushing ages further back in time from these constrained nodes.

Although there appears to be a convergence in the age estimates for angiosperms across different studies, new data (whether molecular or fossil), along with new estimation methods, will help to further refine our ideas of the age and time frame of angiosperm origins and diversification. With the interest in using large angiosperm phylogenies to investigate questions concerning ecology and comparative biology, along with the continual refinement in relationships among major angiosperm lineages, a new estimate of the ages of the major clades is much needed and should be pursued.

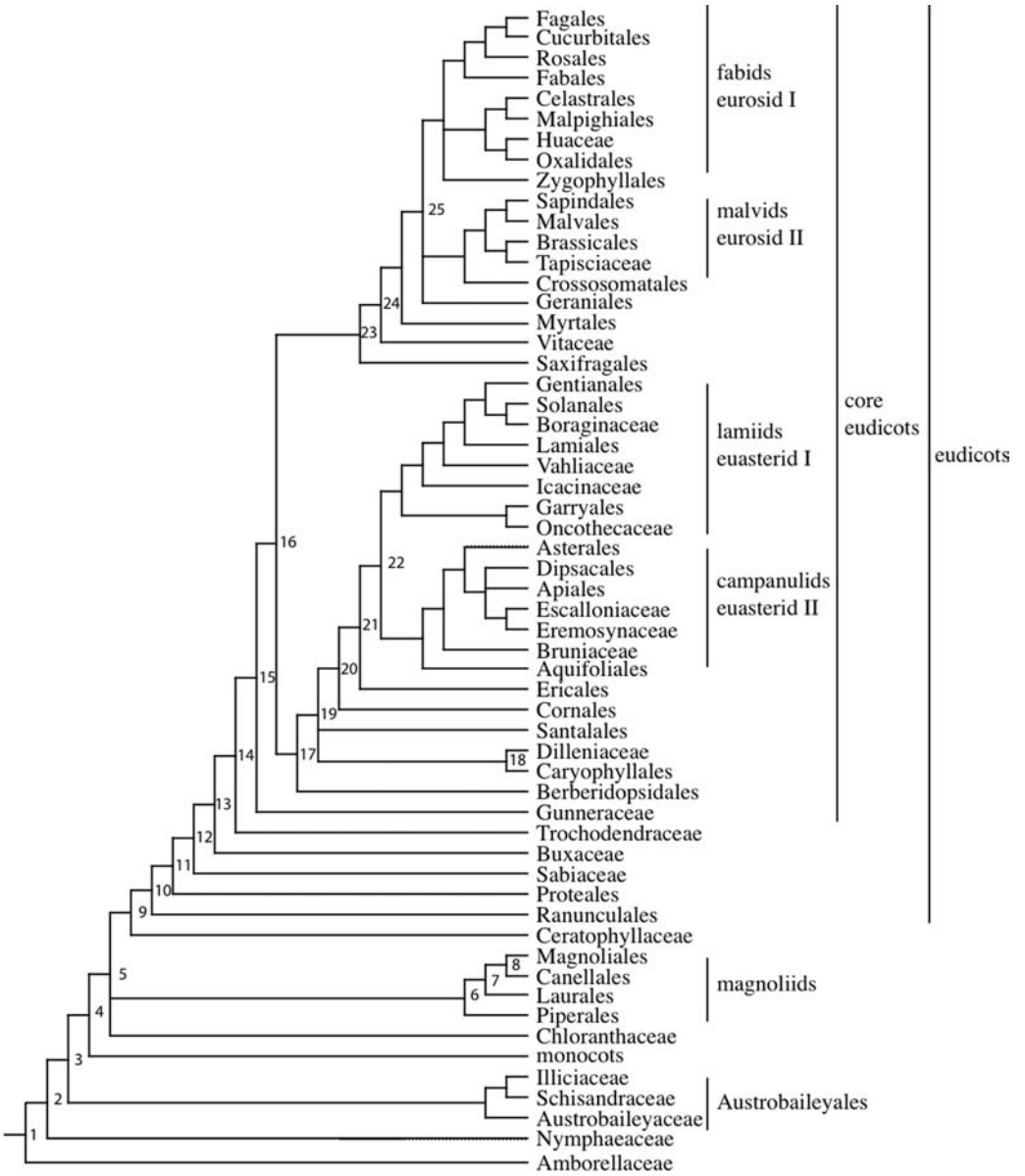

Figure 3. Divergence time estimates for major clades of angiosperms from a reanalysis of the three-gene, 567-taxon data set by using multiple calibration points and methods (see text). Dates corresponding to numbered nodes are given in Table 1.

Extant Members of "Old" Lineages Represent Recent Diversifications

The divergence time estimates of Yoo *et al.* (2005) suggest that crown group Nymphaeales date to the Eocene (44.6–67.9 Ma); Nymphaeaceae and Cabombaceae split at that point. Extant genera of Nymphaeaceae began to diversify in the late Eocene to early Oligocene (41.1–67.7 Ma), and the two extant genera of Cabombaceae diverged during the Miocene (19.9–65.6 Ma). These results indicate that extant Nymphaeales diversified relatively recently, whereas the stem lineage to Nymphaeales is old, on the basis of a fossil attributed to Nymphaeales from the Early Cretaceous (125–115 Ma; Friis *et al.* 2001). Although the Friis *et al.* fossil and a

TABLE 2. Estimated Clade Ages (Myr)

Clade no.[a]	Clade	Wikstrom *et al.*[b]	NPRS[e]	PATHd8[c]	NPRS[d]	PATHd8[d]
1	Angiosperms	158–179	166–187	152–171	165–188	164–173
2		153–171	159–179	132–155	161–180	149–158
3		147–165	151–172	127–142	154–174	138–146
4		—[e]	145–167	125–135	148–170	132–140
5		—	147–165	126–136	150–168	131–140
6		122–132	140–159	45–50	145–162	98
7		127–134	135–153	39–41	139–156	98
8		108–113	118–144	37–39	118–146	98
9		140–155	144–157	123–131	146–160	131–136
10	Eudicots	131–147	135–149	110–119	136–152	125
11		130–144	131–146	108–118	132–149	123–124
12		128–140	130–144	107–117	131–148	122–123
13		124–137	124–139	102–110	125–142	110–116
14		123–135	124–136	100–106	125–139	107–113
15		116–127	118–129	89–95	119–132	96–103
16		114–124	116–124	84–90	117–128	93–96
17		104–111	106–113	84–90	109–117	92–95
18		106–114	107–113	72–77	110–117	86
19		—	104–109	71–76	107–113	79–81
20		102–112	91–104	69–75	96–108	79–80
21		114–125	115–126	86–92	120–130	96–100
22		111–121	113–123	85–89	116–126	94–97
23		108–117	106–120	85–89	109–124	93–97
24		100–109	97–110	84	101–113	87–89

[a]Clade numbers refer to numbered nodes in Figure 3.

[b]Wikstrom *et al.* dates are given in their Supplemental Data, available online. Because we tried to estimate the age of the root, but with constraints as noted, it is possible that a range of solutions might exist. For each analysis, we fixed two ages: (1) the age of the root node (most recent common ancestor of seed plants) at 310 Myr and (2) the age of the most recent common ancestor of Cucurbitales and Fagales at 84 Myr.

[c]Based on single fixed calibration of the most recent common ancestor of Cucurbitales and Fagales at 84 Myr.

[d]Based on 20 minimum age constraints in addition to single fixed calibration of the most recent common ancestor of Cucurbitales and Fagales at 84 Myr.

[e]Node not compatible with tree used.

fossil attributed to Nymphaeaceae from the middle Cretaceous (90 Ma; Gandolfo *et al.* 2004) may be best placed deeper in the angiosperms, there is a distinct gap between the origin of Nymphaeales and its diversification into modern lineages.

These results for Nymphaeales indicating recent diversification in an ancient lineage agree with similar findings for the basal angiosperms Chloranthaceae (Zhang & Renner 2003) and *Illicium* (Illiciaceae; Morris *et al.* 2007). The fossil record indicates clearly that Chloranthaceae represent one of the oldest angiosperm lineages, with unequivocal reproductive structures resembling those of *Hedyosmum* from the Barremian–Aptian boundary, approximately 125 Ma (see Friis *et al.* 1994, 1999; Friis 1997; Doyle *et al.* 2003; Eklund *et al.* 2004). However, divergence time estimates based on molecular data indicate that the extant genera of Chloranthaceae are relatively young (i.e., 60–29 Ma for *Hedyosmum*, 22–11 Ma for *Chloranthus*, and 18–9 Ma for *Ascarina*; Zhang & Renner 2003).

Similar results have been obtained for *Illicium* (Illiciaceae). Morris *et al.* (2007) estimated divergence times within *Illicium* by using penalized likelihood and multiple calibration points. The *Illicium* crown group appears to have arisen

during the Cretaceous; Frumin and Friis (1999) identified *Illiciospermum pusillum* as the first unequivocal evidence of the family, with an estimated age of 89–99 Ma. However, extant New World taxa diversified as recently as the Miocene or Pliocene.

Many Diversifications Occurred in a Narrow Window

Many of the divergences of major clades of angiosperms occurred rapidly. For example, molecular dating techniques provide a time frame for the likely rapid diversification of the five major lineages of Mesangiospermae (magnoliids, monocots, Chloranthaceae, eudicots, Ceratophyllaceae)—this diversification, ultimately yielding perhaps 97% of all angiosperm species, was rapid, occurring over a span of perhaps no more than 5 Myr (Moore *et al.* 2007). In perspective, this span represents a time frame comparable to the rapid radiation of the Hawaiian silversword alliance (Asteraceae–Madiinae), which putatively arose from a North American ancestor 5 Ma (Baldwin & Sanderson 1998; Barrier *et al.* 1999).

But how does the rapid radiation or big bang of major angiosperm lineages (Mesangiospermae) compare to the proposed rapid divergences within other major lineages of life? The fossil record provides evidence for a similar big bang for an early Tertiary (~65 Ma) explosion of modern bird orders (Feduccia 2003) that may have occurred over a time frame of just 5–10 Myr (Feduccia 2003), a somewhat longer time frame than that estimated for the diversification of Mesangiospermae. Interestingly, molecular dating is in conflict with the fossil record and has consistently placed the appearance of modern birds as much older (at least 100 Ma; reviewed in Feduccia 2003).

Fossil evidence similarly has suggested an explosive Tertiary radiation of placental mammals (Novacek 1999; Archibald & Deutschuman 2001; Bloch *et al.* 2007; Wible *et al.* 2007). However, as with birds, a recent analysis of mammals performed with sequence data and a supertree approach again indicates that the radiation of living placental mammals is older, occurring perhaps between 100 and 80 Ma; the "phylogenetic fuses" leading to the diversification of placental mammals were "much longer than previously expected" (Bininda-Emonds *et al.* 2007). That is, the diversification of modern mammals as a clade was not as rapid as previously thought.

However, radiations of lineages within the mammals do appear to have been rapid, on the basis of both molecular and fossil evidence. Klaus and Miyamoto (1991) provided an estimate of approximately 5 Myr for the diversification of the major lineages of pecoran ruminants. These are the large, even-toed, hoofed mammals (all belonging to one eutherian mammal order and one infraorder), and their rapid cladogenesis is supported by both molecular and fossil evidence. Allard *et al.* (1992) provided a similar window for the diversification of the major bovid lineages (bison, cattle, and buffalo), a radiation supported by molecular and fossil evidence.

In contrast to data for all mammals and birds, molecular and fossil data are in close agreement for the timing of both the origin and early diversification of angiosperms (reviewed in Bell *et al.* 2005). Furthermore, the speed (< 5 Myr) and magnitude (~ 350,000–400,000 extant species) of the explosive radiation of flowering plants may be unique.

Subsequent radiations within the clades of Mesangiospermae also occurred rapidly. For example, the Saxifragales and rosid clades also diversified over similar and narrow time spans (Jian *et al.*, 2008; Wang *et al.*, submitted). The rapid diversification within the rosids (Wang *et al.*, submitted) may be of particular importance in that the inferred bursts of diversification correspond in timing with the rapid rise of angiosperm-dominated forests, as suggested by the fossil record (Crane 1987; Upchurch & Wolfe 1993).

The rosid diversification also corresponds to the diversification of several other lineages that apparently evolved in parallel with the

diversification of angiosperm forests (crown group rosids originated 110 [±6] to 93 [±6] Ma, followed by rapid diversification into the eurosid I and eurosid II clades around 108 [±6] to 91 [±6] Ma and 107 [±6] to 83 [±7] Ma): ants (Moreau *et al.* 2006), beetles, and hemipterans (Farrell 1998; Wilf *et al.* 2000). Slightly later diversifications—amphibians (Roelants *et al.* 2007), ferns (Schneider *et al.* 2004), and primates (Bloch *et al.* 2007)—appear to have closely tracked the rise of rosid-dominated forests (Wang *et al.*, submitted). A similar window of diversification is seen in tree species in clades other than the rosids. Cornales of the asterid clade also appear to have diversified during this same window of time (e.g., Bremer *et al.* 2004; Xiang, pers. comm.), as did the woody members of Saxifragales (e.g., Altingiaceae; Jian *et al.*, 2008).

Genome Evolution

Polyploidy has long been recognized as a major evolutionary force in many plant lineages, particularly ferns and angiosperms (Stebbins 1950; Grant 1981; Soltis & Soltis 1999). Although polyploidy is considered common in angiosperms, its actual frequency has been debated. Classic studies using base chromosome numbers estimated that 30%–60% of angiosperms might be of polyploid origin (Muntzing 1936; Darlington 1937; Stebbins 1950; Grant 1981). But recent genomic studies reveal evidence of ancient polyploidy in virtually all angiosperm genomes investigated to date (e.g., Vision *et al.* 2000; Bowers *et al.* 2003; Blanc & Wolfe 2004; Paterson *et al.* 2004), indicating that all angiosperms may have experienced one or more rounds of genome duplication. Analysis of the complete genome sequence of *Arabidopsis* suggested three ancient polyploidy events, one within the Brassicales, as well as two more ancient duplication events; these two more ancient whole-genome duplications were suggested to have occurred (1) before, or coincident with, the origin of the angiosperms and 2) just before or coincident with the divergence of major core eudicot lineages (Bowers *et al.* 2003). From analyses of the complete genome sequence of grape (*Vitis*), Jaillon *et al.* (2007) suggest that the common ancestor of *Vitis*, *Populus*, and *Arabidopsis* was an ancient hexaploid that arose after the monocot-versus-eudicot split. After the formation of this paleohexaploid, there were subsequent genomewide duplication events in the Brassicales and *Populus* lineages (Fig. 4). However, in their independent analysis of the grape genome, Velasco *et al.* (2007) proposed that *Vitis* experienced a more recent whole, or perhaps large-scale, genome duplication event. Velasco *et al.* proposed an alternative scenario: three genomewide duplication events in *Arabidopsis* and *Populus*, one of which was shared by all eudicots (and perhaps the monocots), as well as one duplication event shared by *Arabidopsis* and *Populus*, with a third event specific to each lineage. They suggested that *Vitis* has the genomewide duplication event shared by all eudicots, as well as a lineage-specific event that may be the result of hybridization (Fig. 4). From these initial comparisons of the first complete nuclear genome sequences, genomewide duplications clearly have frequently occurred, although the exact phylogenetic placements of many of these events remain unclear.

Other genomic investigations using large expressed sequence tag (EST) data set indicate that polyploidy has been prevalent in many angiosperm clades (Blanc & Wolfe 2004), including most crops and several basal angiosperm lineages and a basal eudicot (Cui *et al.* 2006). Signatures of whole-genome duplications are present in the water lily *Nuphar* (Nymphaeales), *Acorus* (Acoraceae, the sister to all other monocots), and the magnoliids *Persea* (Lauraceae) and *Liriodendron* (Magnoliaceae)—corroborating evidence for ancient duplication based on isozymes (Soltis & Soltis 1990)—and the basal eudicot *Eschscholzia californica* (California poppy, Papaveraceae), a member of Ranunculales. However, analyses of ESTs from *Amborella*, the sister to all other extant

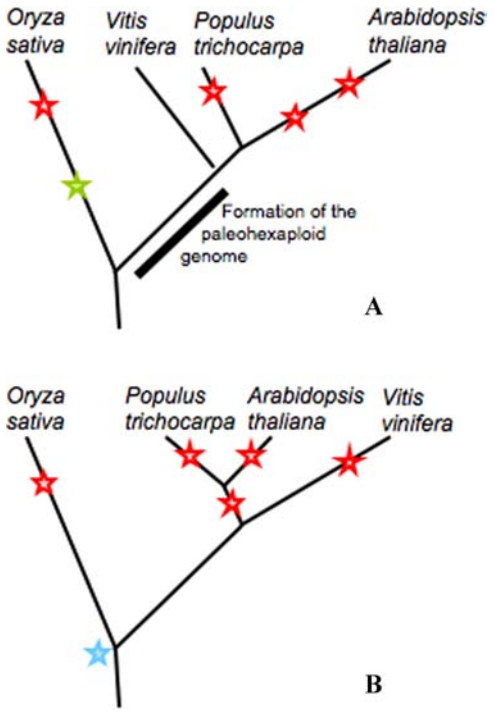

Figure 4. Two hypotheses for genome-wide duplication events in angiosperm evolution on the basis of nuclear genomes sequenced to date. Each star indicates a whole-genome duplication event on that branch. (**A**) Formation of the paleohexaploid ancestral genome occurred after the divergence of eudicots from monocotyledons and before the radiation of the rosids (Jaillon *et al.* 2007); no recent duplication is proposed in grape. Tree redrawn to show *Vitis* sister to all other rosids (Soltis *et al.* 2005). (**B**) Three rounds of duplications are proposed for both *Arabidopsis* and *Populus* (Bowers *et al.* 2003; Macer *et al.* 2005; Tuskan *et al.* 2006; Velasco *et al.* 2007); recent duplication in grape. Yellow star indicates that the duplication event is apparent in the rice genome, but its presence in other monocotyledons is unclear. Blue star indicates a duplication putatively shared by all eudicots (and possibly all monocots). In color in *Annals* online.

angiosperms, have so far not revealed evidence of ancient polyploidy. How many of these whole-genome duplications resulted from shared polyploidization events? Genomic data for additional taxa are needed, but additional evidence for genome duplication is obtainable from analyses of gene families across the angiosperms, without reliance on whole-genome sequences or even large EST sets.

Many crucial genes that control floral initiation and development appear to have been duplicated either just before, or early in, angiosperm evolution; other floral developmental regulators experienced duplication later, near the origin of the core eudicots, a major clade that constitutes 75% of all flowering plants (Kramer *et al.* 2003; Kim *et al.* 2004; Zahn *et al.* 2005; Irish 2006; Kramer & Zimmer 2006). These coincident gene duplications may have resulted from whole-genome duplication rather than independent gene duplications.

Several MADS-box genes, which are crucial regulators of many aspects of plant development, show this pattern of gene duplication. For example, extant gymnosperms have one B-function homologue, whereas all angiosperms have at least two homologues of the *Arabidopsis* genes *AP3* and *PI*, respectively. The two B-function gene lineages, accommodating homologues of *AP3* and *PI,* originated by duplication of one B-function gene before the origin of the angiosperms, perhaps as much as 260 Ma (Kim *et al.* 2004). Another gene duplication event also occurred before the origin of the angiosperms, in the C-function lineage, leading to two lineages in angiosperms, one with *AG* homologues having roles in stamen and carpel identity and the other with ovule-specific D function (Kramer *et al.* 2004). Similarly, *SEP* genes were duplicated to form the *AGL2/3/4* (*SEP1/2/4*) and *AGL9* (*SEP3*) lineages in the common ancestor of the angiosperms (Zahn *et al.* 2005). The corresponding duplications of these key floral organ identity genes before the origin of the angiosperms may have somehow facilitated diversification and innovation of the plant reproductive program, ultimately resulting in the origin of the flower itself (Buzgo *et al.* 2005; Zahn *et al.* 2005). The timing of the C-function and *SEP* gene duplications has not been ascertained, however.

Other MADS gene duplications, including the *APETALA1*, *APETALA3*, *SEP*, and *AGAMOUS* lineages, occurred near the origin of the eudicots (Litt & Irish 2003; Kramer *et al.* 2004; Zahn *et al.* 2005, 2006; Irish 2006).

After the divergence of Buxaceae, the B-function gene *AP3* was duplicated to form the *euAP3* and *TM6* lineages in core eudicots; then the *TM6* gene was lost in several rosids (Kramer *et al.* 1998). Again, the data indicate that MADS duplications might have played an important role in the diversification of the flower.

Still other genes, not directly involved in controlling floral organ identity, also appear to have been duplicated just before the origin of the angiosperms, or near the origin of the eudicot clade. For example, an *rpb2* gene duplication occurred either early in core eudicot evolution or at or near the time of the Buxaceae–Trochodendraceae divergence (Luo *et al.* 2007). The question remains: Were these duplications part of events in which the entire angiosperm genome was duplicated, or were these independent gene duplication events? As the cost of gene and genome sequencing continues to drop, the data will soon be available to address the fascinating hypothesis that whole-genome duplications may have served as catalysts for key innovations in angiosperm evolution.

Floral Developmental Genetics

Although the flower is obviously the central feature of the angiosperms, the origin of the flower and subsequent diversification remain as major evolutionary questions. The ABC model (now often referred to as the ABCE model) has been the unifying paradigm for floral developmental genetics for more than a decade (Coen & Meyerowitz 1991). However, it is based on the phylogenetically derived eudicot model systems *Arabidopsis* (Bowman *et al.* 1989) and *Antirrhinum* (snapdragon) (Schwarz-Sommer *et al.* 1990; Davies *et al.* 2006), with complementary data from other eudicot systems, including *Petunia* (Rijpkema *et al.* 2006) and *Gerbera* (Teeri *et al.* 2006). Multifaceted research collaborations involving phylogenetics, classical developmental studies, genomics, and developmental genetics have recently provided valuable new insights into the early flower and to early angiosperm diversification. We provide an overview here (for more detailed summaries, see Frohlich 2006; Soltis *et al.* 2006, 2007b).

The ABC model posits that floral organ identity is controlled by three gene functions, A, B, and C, that act in combination to produce the floral organs; A-function alone specifies sepal identity, A- and B-functions together control petal identity; B- and C-functions together control stamen identity; C-function alone specifies carpel identity (Fig. 5). Several genes have been identified that act as key regulators in determining floral organ identity in the model eudicots, such as *Arabidopsis* and *Antirrhinum*. For example, in *Arabidopsis*, *APETALA1* (*AP1*) and *AP2* are the A-function genes, *AP3* and *PISTILLATA* (*PI*) are the B-function genes, and *AGAMOUS* (*AG*) is the C-function gene. In *Antirrhinum*, the homologous gene (or homologue) that is comparable to *AP1* is termed *SQUAMOSA*. Details regarding A-function remain complex, however, with A-function not clearly documented except in *Arabidopsis*. The homologues of the A-function gene *AP2* in *Antirrhinum* are *LIPLESS1* and *LIPLESS2*; these may provide partial A-function in snapdragon (Keck *et al.* 2003). The B-function genes in *Antirrhinum* are *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*), which are homologues of *AP3* and *PI*, respectively. The C-function gene in *Antirrhinum* is *PLENA* (*PLE*). All these genes, with the exception of *AP2* (and its homologues), are MADS-box genes (Theissen *et al.* 2000), a broad family of eukaryotic genes that encode transcription factors containing a highly conserved DNA-binding domain (MADS domain).

The ABC model has been updated to accommodate new results, including the identification of additional MADS-box genes that control ovule identity (D-function; Colombo *et al.* 1995) and those that contribute to sepal, petal, stamen, and carpel identity (E-function; Pelaz *et al.* 2000). We will not consider D-function further here because ovules (these become seeds after fertilization) are not discrete floral organs as are sepals, petals, stamens, and carpels.

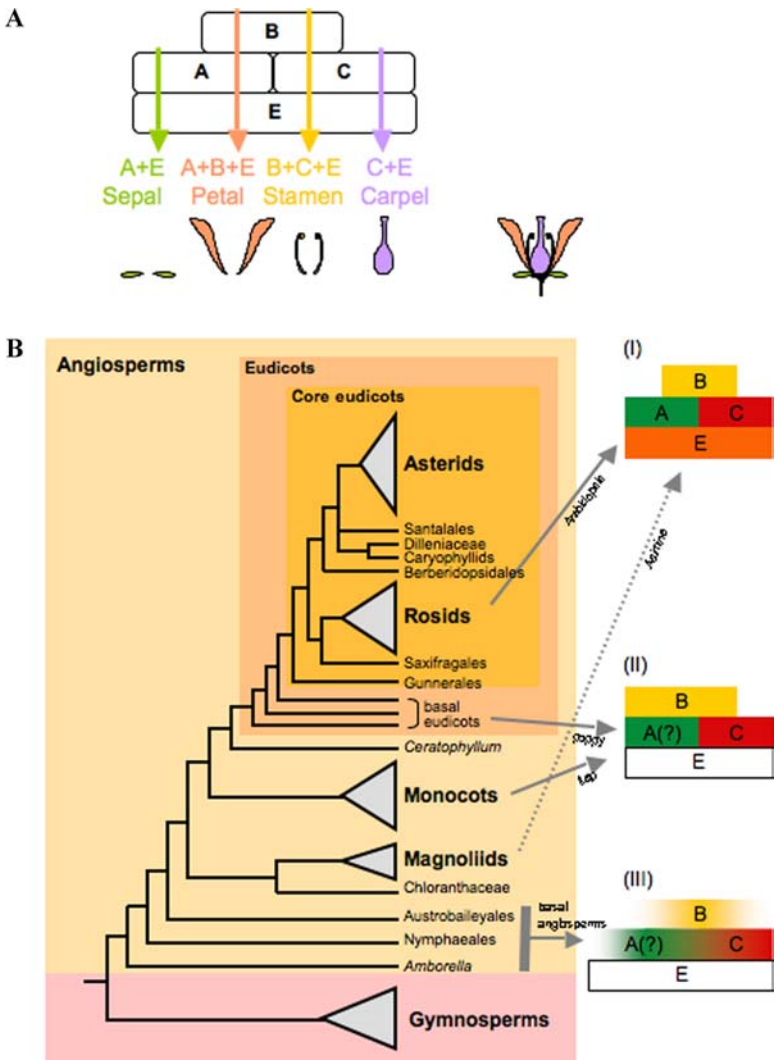

Figure 5. Angiosperm floral developmental genetics. (**A**) Classic ABCE model (Coen & Meyerowitz 1991; Pelaz *et al.* 2000). (**B**) Summarized phylogenetic tree for flowering plants with placements of model organisms. Known or postulated expression patterns are shown on the right for organ identity genes: (I) ABC model developed for core eudicots (Coen & Meyerowitz 1991) may also be applicable to *Asimina*, which is included in the magnoliid clade; (II) an example of the "shifting boundary model" applied to some basal eudicots (Kramer & Irish 2000) and monocots (Kanno *et al.* 2003); (III) "fading borders" model proposed for basal angiosperms (Buzgo *et al.* 2004; Kim *et al.* 2005).

However, E-function plays a major role in the formation of floral organs and is closely allied with ABC functions.

The E-function genes in *Arabidopsis* are *SEPALLATA1* (*SEP1*), *−2*, *−3*, and *−4* (Pelaz *et al.* 2000). SEP proteins, together with the protein products of the ABC genes, are required to specify floral organ identity. The *SEP* genes are functionally redundant in their control of the four floral organ identities—sepals, petals, stamens, and carpels. On the basis of studies in *Arabidopsis*, A + E function is needed for sepals, A + B + E function for petals, B + C + E function for stamens, and C + E function for carpels (Fig. 5). Hence, a more appropriate abbreviation for the current model of floral

organ identity in *Arabidopsis* and *Antirrhinum* is the ABCE model, a designation we use here.

Expression patterns of MADS-box genes in eudicots and grasses typically support the ABC (now the ABCE) model. For example, strong expression of eudicot *AP3* and *PI* homologues is typically limited to petals and stamens, where these genes are required for organ identity specification (Ma & dePamphilis 2000). Studies stemming from the Floral Genome Project (Soltis *et al.* 2002, 2006, 2007; Albert *et al.* 2005) have provided some of the first insights into floral organ identity genes and their patterns of expression in basal angiosperms (Soltis *et al.* 2006). The expression of MADS-box genes in basal angiosperm flowers is generally consistent with the ABC model; however, the expression patterns in basal angiosperms are often broader than expected based on the ABC model (Kim *et al.* 2005). In particular, the homologues of B-function genes, *AP3* and *PI,* are broadly expressed in tepals, stamens, and carpels in many basal angiosperms, including representatives of the three basalmost lineages, *Amborella*, water lilies, and *Illicium* of Austrobaileyales, as well as in members of the magnoliid clade (e.g., *Magnolia*) (Fig. 5).

The floral developmental genetics studies conducted to date for basal angiosperms indicate a broader pattern of expression of B (and to a lesser extent, C and E)-function homologues in basal angiosperms than in eudicots (Kim *et al.* 2005). These results indicate that the ABC model as developed for eudicots is not perfectly applicable to basal angiosperms and, by inference, the earliest angiosperms. The floral morphology of many basal angiosperms provides a crucial hint to what may be a more appropriate model for these plants. In *Amborella* and other basal angiosperms (e.g., *Illicium*), floral organs are spirally arranged, with a gradual transition from bracts to outer and inner tepals, from tepals to stamens, and finally to carpels (Fig. 5). These gradual intergradations of floral organs cannot be easily explained by the classic ABC model and, together with the data obtained from floral developmental studies (briefly reviewed here), resulted in the fading borders model (Buzgo *et al.* 2004, 2005). This model proposes that the gradual transitions in floral organ morphology result from a gradient in the level of expression of floral organ identity genes across the developing floral meristem (Fig. 5). Weak expression at the margin of a gene's range of "activity" overlaps with the expression of another regulator in adjacent cells. This pattern of overlapping expression would result in the formation of morphologically intermediate floral organs rather than organs that are clearly distinct. Recent data from the expression of B-function genes in *Amborella* lend support to this model (reviewed in Soltis *et al.* 2006, 2007b).

The ABC model of floral organ identity is typically considered the default program, with variants viewed as derivatives of this program. In fact, however, when gene expression profiles of floral-organ regulators are compared in a phylogenetic context, the ABC model of *Arabidopsis* is clearly evolutionarily derived. The ancestral flower had broad expression patterns of at least B-function regulators; broad and overlapping expression yielded morphologically intergrading floral organs, as seen in several extant basal angiosperms. Restriction of expression (and function) to specific regions of the floral meristem resulted in the discrete whorls of morphologically distinct floral organs that together characterize most of the eudicots and certainly all the core eudicots. Further investigation of the evolution of the floral regulatory network should rely on the phylogenetic perspective, which reveals that the ABC model is derived.

But how was the genetic machinery necessary for specifying a flower assembled in the first place? Were the genes co-opted from other processes and integrated into a pathway gradually, or were they brought together more suddenly, perhaps through gene or whole-genome duplication? Parallel duplications of floral regulatory genes suggest whole-genome duplications in the common ancestor of extant angiosperms and the common ancestor of core eudicots.

However, the mere duplication of a genome or set of genes was probably not coincident with the origin of morphological novelty. Certainly some time would have been needed for the assembly of a functional floral-organ specification program in the ancestral angiosperm. Furthermore, the duplication of the B-function homologues (*AP3* and *PI*) apparently occurred approximately 260 Ma, 130 Myr before the first fossil evidence of angiosperms. The process of assembling a new genetic program and its translation into morphological innovation merits further study.

Studies of floral developmental genetics have also provided more insights into the evolution of the perianth. The classical view of angiosperm flower evolution maintains that stamens and carpels evolved just once, whereas the sterile perianth organs may have arisen multiple times (e.g., Eames 1961; Takhtajan 1991). This reasoning is based on the idea that angiosperms are derived from apetalous ancestors and that the perianth is an evolutionary novelty. The resemblance of sepals to foliar bracts and of petals to stamens has encouraged the view that sepals are evolutionarily derived from foliar bracts and petals, from stamens. The longstanding view is that such stamen-derived petals, called andropetals (Takhtajan 1991), are associated primarily with eudicots. The perianth of basal angiosperms typically consists of morphologically similar organs, termed tepals (Endress 2001), which could be assigned bracteal (bracteopetal) or staminal (andropetal) origins depending on whether sepallike or petallike features prevail. By these criteria, the tepals of Lauraceae have been considered bracteopetalous (Albert *et al.* 1998; de Craene *et al.* 2003). However, expression data for *Persea* (based on reverse transcription–polymerase chain reaction, fluorescence *in situ* hybridization, and microarrays), coupled with developmental data, suggest that the "petals" of *Persea* and other Lauraceae clearly are of staminal origin (Chanderbali *et al.* 2006, in prep.). These data clearly document that, as long suggested, not all "petals" are homologous. It will be of interest to examine the origin of petals in other basal lineages by using molecular data.

Synthesis, Pragmatism, and the Future

During the past decade, we have witnessed dramatic changes in perspective of some fundamental aspects of the origin and early evolution of flowering plants. For example, the anthophyte hypothesis has come and gone—and possibly come back again in a resurrected form. Likewise, the view of the first flower has changed from something large and *Magnolia*-like to a diminutive form that performed the key function of the flower—reproduction—but that only vaguely resembled most modern flowers. These complex changes in our collective views have been triggered by syntheses of phylogenetics, paleobotany, developmental biology, and genetics. Such broad changes of thought rarely emerge without multidisciplinary synthesis, and our understanding of angiosperm evolution has benefited from a general attitude of collaboration and cooperation among scientists in different disciplines.

Despite this progress, some issues will remain difficult to resolve: What is really the sister group of the angiosperms? How was the genetic machinery that underlies the flower assembled? What role has polyploidy played in the origin and diversification of the angiosperms? What geological and biological factors stimulated angiosperm radiations, and what effects did these radiations have on the rest of Earth's biota? Although difficult, these questions can likewise be addressed through collaboration and multidisciplinary study and with patience. The next decade—perhaps the "postphylogenetic period"—holds tremendous opportunities for advancing our understanding of the events and processes that shaped the origin and early evolution of angiosperms.

New discoveries in paleobotany—through new fossils, new methods, and new interpretation (e.g., Friis *et al.* 2007)—will continue to revise and enhance our understanding of

angiosperm origins and early evolution. Studies of codiversification among major clades of life, coupled with improved understanding of climatic events in Earth's history, will lead to new views of the processes that build communities and ecosystems and how those processes have shaped today's biota. Finally, genomic analysis of nonmodel systems holds countless clues to the origin and early diversification of angiosperms. For example, ongoing genomic studies of phylogenetically key basal angiosperms (*Amborella*, *Nuphar*, *Persea*, *Liriodendron*, and *Aristolochia*) are providing crucial data for inferring the role of polyploidy in early angiosperm evolution (http://www.ancestralangiosperm.org). Eventually, complete genome sequences for one or more of these early angiosperms will enable analyses of polyploidy, genome evolution, and the assembly of genetic pathways and networks. With rapid advances in next-generation sequencing techniques and their application to comparative genomics (e.g., Moore *et al.* 2006; Wall *et al.* submitted), genome sequences for evolutionarily important species may soon be forthcoming. The *Amborella* nuclear genome is an extremely strong candidate for sequencing (Soltis *et al.* 2008). Not only is *Amborella* the sister to all other extant angiosperms, thus providing an evolutionary reference for all other studies, but genomic resources are already in place.

Conflicts of Interest

The authors declare no conflicts of interest.

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